IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Allison M. Ford
Art Unit	:	1651
Applicants	:	Francois Romagne, Catherine Laplace
Serial No.	:	10/505,252
Filed	:	August 19, 2004
Conf. No.	:	7415
For	:	Methods for Producing Gamma Delta T Cells

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

DECLARATION OF SAMUEL SALOT UNDER 37 C.F.R. §1.132

Sir:

I, Samuel Salot, hereby declare:

THAT, I have been employed as a Research and Development Project Manager at Innate Pharma, S.A. in Marseilles, France since November 2002;

THAT, I have been responsible for the project management of laboratory immunomonitoring, cell therapy projects, various immunology projects and cell culture projects, including a cell culture project falling within the scope of the claims of application Serial No. 10/505,252 (hereinafter "the '252 application"), filed August 19, 2004;

THAT, I have been a co-author on numerous papers related to culture systems and methodologies for the selective enrichment of γδ T-cells, as evidenced by my attached Cirriculum Vitae;

THAT, I have read and understood the '252 application, the Office Actions dated April 4, 2006, February 8, 2007, and July 31, 2008 and the references cited in those Office Actions; and

Being thus duly qualified, I declare as follows:

1. Claims 21, 23-25, 27-34 and 51-52 have been rejected under 35 U.S.C. § 103(a) in the Office Action dated July 31, 2008 on the grounds that the claimed invention is obvious over Belmant *et al.*, U.S. Patent No. 6,660,723), in view of Skea *et al.*, Garcia *et al.* and Valeri *et al.*

2. In the Office Action, it is argued that the initial number of cells utilized in a cell culture protocol is generally recognized to be a result effective variable that directly affects the final number of cells produced by the culture. The Office Action further argues that depending upon the scale of the culture to be carried out, the cell count of the initial biological sample would have been routinely optimized to provide the desired concentration of cells (cells/mL) and that this concentration of cells is more important than the total number of cells used in the culture protocol. The Office Action further notes that Belmant *et al.* is silent with respect to the concentration at which cells are maintained throughout the culture period; however, it is argued that Skea *et al.* is directed to the culture of T-lymphocytes and teaches the culture of cells at an initial concentration of 1×10^5 cells/mL and passaging of the cells every 4-7 days at that same concentration (Skea *et al.* at page 526, column 1, "T Cell Culture").

The Office Action further argues that it was recognized that the duration of culture was recognized as directly affecting the final percentage of γδ T-cells in a culture. In this regard, the Office Action relies on the teachings of Skea *et al.* which indicate that the percentage of γδ T-cells increased linearly over time, up to 27 days (Skea *et al.* at page 534, Figure 7) and that this teaching supports the assertion that the duration of culture is a result effective variable that directly affects the degree of enrichment of γδ T-cells.

3. Regarding these arguments, I note that the argument advanced in the Office Action is incorrect as it is recognized that cell concentrations of greater than five (5) million viable cells per

mL in a culture system such as that employed by Belmant *et al.* or Espinosa *et al.* would not be employed because a majority of the cells would be expected to die when cultured at such numbers. While some cells might stay alive, but the vast majority of cells would not find sufficient amounts of nutrients or space to grow efficiently. Additionally, γδ T-cells are very minor fraction of the total cell number at the start of culture (less than 5%) and reach a very high proportion (> 70%) within few days (around 7 days) of stimulation with compound that activates γδ T-cells. Furthermore, cell to cell contact is required from day 0 on to successfully expand γδ T-cells; however, if too high a concentration of cells (> 5 million / ml) exists, these cells will encounter limited space and a lack of necessary nutrients that will not favor their survival. Thus, I would not agree with the position that the concentration of cells would be optimized to the claimed levels as argued by the Office Action.

4. The Office Action also argues that Skea *et al.* teach the passaging of cells at a particular cell number as well as a recognition that the duration of culture was recognized as affecting the total percentage of γδ T-cells at the end of the culture period. In this regard, I note that Skea *et al.* is directed to the selective expansion of functional T-cell subsets using a conditioned medium denoted as XCLM™. As noted at page 526, XCLM™ is a conditioned medium prepared from whole umbilical cord blood. This conditioned medium is substantially different from that utilized in the instant cell culture protocol, particularly with respect to the amount of IL-2 that is used in the culture of the cells (about 2 ng/mL in XCLM™ as compared to about 21.4 ng/mL in the disclosed protocol at page 25 of the as-filed specification and about 9.2 to 30.5 ng/mL in the claimed protocol [see claim 33]).

I also note that Skea *et al.* indicate that cultures of both umbilical cord blood and adult peripheral blood T-cells treated with 10 ng/mL IL-2 expanded less than 10-fold and not beyond one week (7 days; see page 535, column 1, first paragraph) when cultured in medium similar to that used in the instantly claimed culture protocols. Thus, contrary to the arguments advanced in the Office Action, one skilled in the art would not have recognized that culturing T-cells in serum free medium and in the presence of IL-2 was a result effective variable that would have resulted in an increased percentage of γδ T-cells; rather, one skilled in the art would have expected that culturing T-cells in

serum free medium containing IL-2, at levels such as that claimed in this application, would have resulted in maximal proliferation having occurred with seven (7 days) after the T-cells were first cultured.

5. With respect to the IL-2 concentrations discussed above, the following calculation was performed to arrive at those values, using the art recognized conversion factor of 1.1mg IL-2 equaling 18 million IU:

350 U/ml * 1.1 mg/18,000,000 U * 1,000,000 ng/mg = 21.4 ng/mL
150 U/ml * 1.1 mg/18,000,000 U * 1,000,000 ng/mg = 9.2 ng/mL
500 U/ml * 1.1 mg/18,000,000 U * 1,000,000 ng/mg = 30.5 ng/mL

6. Claims 21, 23-25, 27-34 and 51-52 are also rejected as being unpatentable over Espinosa *et al.*, in view of Skea *et al.*, Garcia *et al.* and Valeri *et al.* Both Skea *et al.* and Garcia *et al.* appear to be relied upon for very similar reasons as set forth in the rejection discussed above. To the extent that Skea *et al.* is relied upon in such a fashion, my discussion of the reference is also applicable to this rejection. Namely, the selective expansion of functional T-cell subsets in that reference used a conditioned medium denoted as XCLM™ that is a conditioned medium prepared from whole umbilical cord blood and which is substantially different from that utilized in the instant cell culture protocol, particularly with respect to the amount of IL-2 that is used in the culture of the cells. Further, Skea *et al.* indicate that cultures of both umbilical cord blood and adult peripheral blood T-cells treated with 10 ng/mL IL-2 expanded less than 10-fold and not beyond one week (7 days; see page 535, column 1, first paragraph) when cultured in medium similar to that used in the instantly claimed culture protocols.

Thus, contrary to the arguments advanced in the Office Action, one skilled in the art would not have recognized that culturing T-cells in serum free medium and in the presence of IL-2 was a result effective variable that would have resulted in an increased percentage of γδ T-cells; rather, one skilled in the art would have expected that culturing T-cells in serum free medium containing IL-2, at

levels such as that claimed in this application, would have resulted in maximal proliferation having occurred with seven (7 days) after the T-cells were first cultured in view of the explicit teachings of Skea *et al.*

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By: _____
Samuel Salot

31st October 2008
Date

Attachment: Cirriculum Vitae

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R&D Project Manager, Engineer, Scientific contracts & External collaborations

Work experience

Since 11. 2002	R&D Project Manager, Innate Pharma, Marseilles, France *immunomonitoring laboratory project Management, Cell therapy project Management, Development of collaboration with academic partners, Management of several projects in immunology (pharmacology, Cell culture, etc...)*
2001-2002 1 year	Technician, Cancer institute, Rennes, France *Haematology, radio-immunology, biochemistry, etc...*
2000-2001 1.5 years	Technician, Cancer institute, Angers, France *Flow cytometry, anatamopathology, radio-immunology*

Formation

2003-2007	Diplôme de l'EPHE (three-year), Master Degree in the *Ecole Pratique des Hautes Etudes* (EPHE), Paris
1998-2000	BTS in Biochemistry (two-year) at *Lycée Jean-Perrin*, Nantes.
1998	Baccalauréat (French equivalent of A level)

Bibliography

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Laplace C., Saïagh S., Tenaud I., Salot S., Bennouna J., Bonneville M., Romagne F., Tiollier J. Innacell GD: Process to manufacture specific Vγ9Vδ2 T lymphocytes cell therapeutic product from peripheral blood mononuclear cells (PBMC) using bromohydrin pyrophosphate (BrHPP) and Interleukin 2 (IL-2). AACR meeting (Orlando, march 2004)